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Effect of *Lactobacillus plantarum* on Growth Performance and Prevention of *Escherichia coli* Infection in Native Chickens

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This study evaluated the effects of *Lactobacillus plantarum* as a probiotic on growth performance, feed efficiency, and resistance to *Escherichia coli* (*E. coli*) O1 infection in local chickens. Chicks were orally challenged with *E. coli* O1 within 24 hours post-hatch and divided into four groups: a control group (no probiotic) and three treatment groups supplemented with *L. plantarum* at 0.1, 0.2, and 0.3%, respectively. All groups received identical diets and had *ad libitum* access to feed and water. Supplementation at 0.2 and 0.3% significantly ($p < 0.05$) improved body weight and feed conversion ratio, particularly during the second grower phase. The 0.3% group also showed enhanced disease resistance, with shorter infection duration, higher survival rates, and significantly reduced *E. coli* counts in feces, ileum, and cecum ($p < 0.05$). These findings suggest that *L. plantarum*, particularly at 0.3%, is effective in promoting growth and improving gut health in chickens challenged with *E. coli*.
Keywords: Poultry, *Lactobacillus plantarum*, *Escherichia coli*, probiotic, growth performance, disease prevention.

INTRODUCTION

Poultry farming is an essential component of global agriculture, significantly enhancing the production of protein-rich foods. Nevertheless, the sector has numerous challenges, primarily widespread bacterial infections, particularly *Escherichia coli (E. coli)*, which can result in significant economic losses. *E. coli* commonly infects poultry, causing many infectious diseases that affect the digestive system of chickens (Panth, 2019). Excessive use of antibiotics or antibiotic-containing products leads to antibiotic residues in poultry farming, reducing treatment effectiveness, and affects the health of consumers (Rahman & Hollis, 2023; Albaayit, 2021). Therefore, finding alternative products is necessary for poultry farming today.

Probiotics are widely used to improve poultry health, specifically *Lactobacillus plantarum* (*L. plantarum*). *L. plantarum* is a beneficial bacterium and has been extensively studied for its role in improving gut microbiota, enhancing immune responses, and inhibiting the growth of pathogenic bacteria (Al-Shawi et al., 2020; Manin et al., 2024; Sharma et al., 2018) such as *E. coli*. Probiotic supplementation with *L. plantarum* has been shown to improve growth performance, feed efficiency and disease resistance in poultry by balancing the intestinal microflora. Particularly Swelum et al. (2021) debated that chickens can die after 5 days of *E. coli* infection. However, Treating *E. coli* infections in poultry remains a significant challenge. Conventional methods are often ineffective and can disrupt the delicate balance of the gut, stunt growth and driving up farming expenses. Compounding the problem, these bacteria are highly virulent and pose a zoonotic risk, meaning they can transmit from birds to humans (Pokharel et al., 2023). In search of a sustainable alternative, many researchers have turned to probiotics. Studies indicate that these beneficial bacteria can profoundly improve poultry health by enhancing digestion, boosting growth rates, and strengthening the immune system. This often leads to a lower incidence of gut diseases and more efficient feed conversion, which translates to better economic outcomes for farmers (Amara & Shibl, 2015; Ari et al., 2024). Among the most promising strains is *Lactiplantibacillus plantarum*. It is known to suppress harmful pathogens while simultaneously helping animals better digest and absorb nutrients (Yin et al., 2023). By stimulating enzyme activity in the gut, probiotics like *L. plantarum* increase the nutritional value of feed (Sharma et al., 2018). Crucially, it offers a natural defense, bolstering the host's immunity and improving overall productivity without contributing to the growing crisis of antibiotic resistance (Al-

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Shawi et al., 2020). Furthermore, it can create a barrier against pathogenic invasion and help curb the proliferation of drug-resistant bacterial strains (Kuebutornye et al., 2020; Nurfauziah et al., 2024). This research is particularly urgent for Vietnam, where the poultry industry is rapidly evolving from small, family-run farms to large-scale commercial operations. This intensification has, unfortunately, been accompanied by the swift spread of infectious diseases like avian colibacillosis (Al-Shawi et al., 2020; Manin et al., 2024; Sharma et al., 2018). Our study is the first to evaluate the efficacy of *L. plantarum* specifically in Noi chickens, a prized native breed. Moving beyond previous work focused on industrial hybrids (Amara & Shibl, 2015; De Cesare et al., 2017; De Souza et al., 2018; Sharma et al., 2018; Wang et al., 2023; Qui et al., 2024; Ari et al., 2024), we test the practical application of this probiotic within the context of local Vietnamese farming. We provide a detailed analysis of how it reduces *E. coli* levels throughout the digestive tract—in the ileum, cecum, and feces—offering a viable strategy to reduce dependence on antibiotics. Ultimately, this study aims to demonstrate how *L. plantarum* can serve as a effective probiotic to curb *E. coli*, enhance growth performance, and fortify disease resistance in local poultry.

MATERIALS AND METHODS

***Location*:** The experimental isolation and selection of probiotic bacteria were conducted at the Animal Biotechnology Laboratory, Faculty of Animal Husbandry, College of Agriculture, Can Tho University, and Tra Vinh University Laboratory. The potential bacteria were confirmed using 16S rRNA gene sequencing of *Lactobacillus* spp. at T&N Biosolution Company Limited (Vietnam). The experiment was conducted at the poultry farm in a rural household located in An Chay hamlet, Thanh My commune, Chau Thanh district, Tra Vinh province from November 2022 to May 2023. The study was conducted following the Animal Husbandry Law of Vietnam (32/2018/QH14) and the guidelines approved by the Council for Science and Education, College of Agriculture (A10-02-2019/KNN), Can Tho University.

***Infected bacteria preparation*:** In the current study, a commercial product of *E. coli* (ATCC® 49223TM) was used at a concentration of $10^{7.9}$ CFU/mL (Lethal dose 50 determination), which was calculated according to the formula from the previous study (Reed and Muench, 1938).

Probiotic isolation: The samples for probiotics were collected from chicken intestines at the local chicken farm in Can Tho city, Vietnam. The isolation of *Lactobacillus* spp. followed the protocol of Vietnam (TCVN 8737 – 2011). The CTRL9 strain was screened for probiotic characteristics (Nam et al., 2022). After evaluated the probiotic potential and performing molecular identification through PCR, 6S rRNA gene sequencing was used to confirm CTRL9. The primer set was based on the results of the previous study (Kim et al., 2020)
16S–23S CCT TTC TAA GGA AGC GAA GGA T 129 0.4
Acidophilus–R ACG CTT GGT ATT CCA AAT CGC
The BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to compare the 16S rRNA gene sequence with the database on GenBank. The results showed that the homology level was 98.34%. The bacteria were identified as *L. plantarum.*

Preparation of probiotics powder: The preparation process was carried out based on the research from previous study (Vidhyasekaran & Muthamilan, 1995):
Carrier: the study used the IR5040 rice variety, which was finely ground to form rice bran as a carrier. The carrier (rice bran) was distributed into plastic bags (2.5 g/bag) and sterilized. Bacterial suspension: A rod was used to take a single colony from a Petri dish after 24 hours of incubation and placed into a falcon tube containing 10 ml of MRS medium (De Man, Rogosa, Sharpe, M641–500G). The tube was shaken at 150 rpm for 24 hours. After 24 hours, 1 ml of bacterial was transferred into a 250 mL conical flask and shaken again at 150 rpm for 24 hours
L. plantarum incorporation into the carrier: bacteria cultured after 2 days were measured for absorbance (OD) at a wavelength of 600 nm. Then, based on the standard curve, the bacterial suspension was mixed to a density of 10^7 – 10^8 CFU/g. A volume of 1 mL of bacterial suspension was injected into each carrier bag and thoroughly mixed to ensure an even distribution of bacterial cells.
Add *L. plantarum* strain to the carrier: bacteria cultured for 24 hours were assessed for *L. plantarum* cell density by planting them on an agar plate before proceeding with inoculation into the carrier. The ratio of bacterial suspension/carrier (1 ml/2.5 g of carrier) was mixe, ensuring an even distribution of bacterial cells. These operations are conducted in a sterile incubator. Then, the prepared bags are placed and dried at 40^{o}C. Every 6 hours, the moisture content was measured using a moisture balance. After drying, the preparation achieved a moisture content of less than 20%, meeting the required standards. The density of surviving cells was determined by diluting the sample and inoculating it onto an agar surface to count count colonies and calculate CFU/g. The final preparation was stored at room temperature (29^{o}C ± 2^{o}C).

***Chick management and diet*:** The one-day-old native broiler chicks (named Noi chicken in Vietnamese) were utilized in this study. All chicks were immunized against coccidiosis (on days 1 and 3), Newcastle disease (on days 5, 19, and 42), Gumboro disease (on days 7, 14, and 21), and highly pathogenic avian influenza (on day 15). A commercial, antibiotic-free feed was used as the basal diet and was provided equally to individual pens at 7.00 AM and 2.00 PM throughout the experiment. The chemical composition of the basal diet is presented in Table 1.

Table 1. The chemical compositions of a basal diet for 1-45 days old chicks in the current study.

Chemical composition	Periods (weeks)	
	2 – 5	6 – 10
Metabolisable energy (Kcal/kg) (min)	2.850	3.000
Crude protein (%) (min)	20.0	18.0
Crude fiber (%) (max)	5,0	6,0
Calcium (%) (min–max)	0.6 – 1.2	0.6 – 1.2
Available phosphorus (%) (min–max)	0.5 – 1.0	0.5 – 1.0
Digestible lysine (%) (min)	1.1	1.0
Digestible Methionine + Cysteine (%) (min)	0.8	0.75

Evaluation of the protective effect of probiotics to prevent E. coli in broiler chicks: A completely randomized design was used with 96 one-day-old native chicks, divided into four treatment groups, each with four replicates of six chicks. The dietary treatment groups were as follows: control group (P0.0): basal diet only; treatment 1 (P0.1): basal diet + 0.1% probiotic + *E. coli* O1; treatment 2 (P0.2): basal diet + 0.2% probiotic + *E. coli* O1; treatment 3 (P.03): basal diet + 0.3% probiotic + *E. coli* O1.

The probiotics were mixed into the feed daily from day 1 until the end of the experiment. *E. coli* infection was performed only once when the chickens were 2 days old by administering 1 mL of *E. coli* suspension at a concentration of $10^{7.9}$ CFU/mL. After 24 hours of infection, fecal samples were collected to check for the presence of *E. coli* O1 bacteria. Subsequent fecal samples were collected weekly to monitor *E. coli* O1 level. At the end of the experiment, one chicken per experimental pen was euthanized to check for E. coli in the intestines. Samples from ileal, cecal, and fecal contents were collected to determine *E. coli* bacterial density (CFU/ml).

Chickens were weighed before the experiment began and in the morning before feeding. Chickens at the age of 1–3 weeks were weighed using an Ohaus scale (USA) with a minimum accuracy of 0.1 grams, while those older 3 weeks were weighed using a digital scale (Vietnam) with a minimum division of 2 grams. The chickens after 7 days were weighed once in the morning until the end of the experiment. Feed consumption was calculated based on the difference between feed intake and feed leftovers after 7 days.

Survival rate (%) = (Number of surviving chickens after the experiment / Total number of chickens at the start) x 100.

$$\text{Disease rate (\%)} = \frac{\text{Number of infected chickens}}{\text{Total number of chickens at the start}} \times 100$$

Data analysis: The collected data were processed using Microsoft Excel 2016 and statistically analyzed using ANOVA in Minitab version 16.2.1 software (State College, PA, USA). A *p*–value lower than 0.05 was considered statistically significant.

RESULTS

Effects of probiotics on the growth ability of local chickens: The data showed that *L. plantarum*, especially at concentrations of 0.2% and 0.3%, had a positive effect on the growth performance and feed utilization of chickens during both growth phases (1–28 days and 29–45 days) (Table 2). During the first phase, no significant differences were observed in body weight, daily gain, or feed intake among

Table 2. Effects of probiotics on growth and feed metabolism of chickens.

Criteria	Treatments				SEM	P
	$P_{0.0}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$		
Stage 1–28 days old						
Weight at 1 day old (g)	31.76	30.92	31.08	31.92	0.74	0.725
Weight at 28 days old (g)	221	211	212	222	2.45	0.125
Daily weight gain (g/d)	7.03	6.67	6.72	7.04	0.15	0.204
Feed intake (g/d)	16.87	15.60	14.85	15.60	0.48	0.072
FCR	2.40^{a}	2.34^{ab}	2.21^{b}	2.21^{b}	0.04	0.006
Stage 29–45 days old						
Weight at 29 days old (g)	221	211	212	222	2.45	0.125
Weight at 45 days old (g)	428^{b}	421^{b}	440^{a}	455^{a}	3.80	0.001
Daily weight gain (g/d)	12.18^{b}	12.37^{b}	13.40^{a}	13.76^{a}	0.16	0.001
Feed intake (g/d)	37.69	37.56	37.83	37.88	0.14	0.394
FCR	3.10^{a}	3.04^{a}	2.82^{b}	2.76^{b}	0.04	0.001

a, b, c: Different characteristics within the same row exhibited a statistically significant difference (P<0.05).

Table 3. Effect of probiotics on disease prevention ability of chickens .

Items	Treatments				SEM	P
	$P_{0.0}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$		
Stage 1–28 days old						
Number of days infected chickens/number of days raised (days)	0.00^{c}	8.93^{a}	7.14^{ab}	3.57^{bc}	1.15	0.001
Survival rate (%)	100^{a}	75^{c}	80^{bc}	90^{ab}	3.23	0.001
Stage 29–45 days old						
Number of days infected chickens/number of days raised (days)	0.00^{c}	5.88^{a}	4.41^{ab}	1.47^{bc}	1.04	0.007
Survival rate (%)	100^{a}	83.48^{b}	90.53^{ab}	97.5^{a}	2.49	0.002

Noted: a, b, c: Different characteristics within the same row exhibited a statistically significant difference (P<0.05). $P_{0.0}$ was not infected by E. coli O1.

Table 4. Density of *E. coli* O1 between treatments of local chickens (log10 CFU/ml).

Item		Treatments				SEM	P
		$P_{0.0}$	$P_{0.1}$	$P_{0.2}$	$P_{0.3}$		
Stage 1–28 days old							
Feces	*E. coli*	0.00^{c}	5.59^{a}	5.34^{ab}	5.16^{b}	0.09	0.0001
Stage 29–45 days old							
Feces	*E. coli*	0.00^{c}	5.19^{a}	4.98^{ab}	4.50^{b}	0.15	0.0001
Ileum	*E. coli*	0.00^{c}	4.41^{a}	4.17^{a}	3.86^{b}	0.07	0.0001
Cecum	*E. coli*	0.00^{c}	4.10^{a}	3.75^{b}	3.58^{b}	0.08	0.0001

Noted: a, b, c: Different characteristics within the same row exhibited a statistically significant difference (P<0.05). $P_{0.0}$ was not infected by E. coli O1.

treatments. However, the feed conversion ratio (FCR) was significantly increased in the P0.2 and P0.3 groups compared to the control and P0.1 groups. In the second phase, body weight and daily gain were significantly higher in the P0.2 and P0.3 groups, while the FCR was significantly lower, indicating better feed utilization when higher levels of probiotics were supplemented.

Effects of probiotics on disease prevention ability of local chickens: The data in Table 3 indicated that P0.3 group had the greatest impact on overall health and *E. coli* resistance in chickens. During the first phase, chickens that received higher levels of *L. plantarum* (0.2% and 0.3%) exhibited a shorter infection duration, and higher survival rates compared to the P0.1 group. A similar trend was observed in the second phase (29–45 days), where probiotic treatments further reduced infection rates and increased survival, particularly in the P0.3 group.

Effect of probiotics on E. coli density: Table 4 presents the effects of *L. plantarum* supplementation on *E. coli* colonization in the gut. The results showed that dietary *L. plantarum* significantly decreased *E. coli* counts in feces, ileum, and cecum during both growth phases. In the P0.1 group, *E. coli* showed significantly higher. *L. plantarum* supplementation, especially at 0.2% and 0.3%, effectively reduced *E. coli* populations in all targeted criteria, with the lowest levels observed in the 0.3% *L. plantarum* group.

DISCUSSION

The findings of the current study demonstrate that *L. plantarum* significantly improves the growth performance of chickens by increasing body weight and enhancing FCR. The observed weight gain is likely a direct result of how *L. plantarum* reshapes the gut environment. This probiotic strain produces lactic acid, which lowers intestinal pH to create an unfavorable environment for harmful bacteria while simultaneously promoting the growth of beneficial microbes (Rangan & Hang, 2017; Wang et al., 2023; De Cesare et al., 2017). This rebalanced microbiota enhances overall digestive efficiency. Furthermore, *L. plantarum* boosts the activity of key digestive enzymes—protease, amylase, and lipase—which improves the breakdown and absorption of nutrients (Wang et al., 2023). The benefits extend beyond digestion; *L. plantarum* also generates short-chain fatty acids (SCFAs) that improve immune function, increase intestinal villus size, and reduce gut inflammation. This allows more energy to be directed toward growth rather than fighting low-grade infection (Sunkara et al., 2012).

In addition, the production of SCFAs can lead to changes in the intestinal microbiota of chickens, which are related to inflammatory responses in the body, so *L. plantarum* also reduces inflammatory responses by reducing the levels of harmful bacteria. More specifically, SCFAs indirectly affect the intestinal bacterial barrier by promoting the secretion of mucin and antimicrobial peptides in the intestine (Sunkara et al., 2012). From there, they have the ability to increase the growth performance of chickens. In addition, the ability to improve FCR was recorded in groups supplemented with higher levels of probiotics, indicating that chickens needed less feed to gain the same amount of weight, which may be due to improved efficiency of digestion, absorption and

utilization of nutrients. Research indicates that *L. plantarum* supplementation enhances poultry growth and health through multiple interconnected mechanisms. Jäger et al. (2018) demonstrated that probiotics, including *L. plantarum*, modulate digestive enzyme activity, thereby improving intestinal nutrient breakdown and absorption, which directly supports animal development. Concurrently, its ability to mitigate inflammatory substances allows chickens to bolster their immunological response, leading to healthier birds and accelerated growth (Wang et al., 2023).

A key benefit of *L. plantarum* is its significant capacity to reduce pathogenic *E. coli* load in the intestine and feces, showcasing its potential as an effective probiotic for harmful bacterial control (Wang et al., 2017). It achieves this through a multi-faceted approach: competitively excluding pathogens for nutrients and adhesion sites on the intestinal mucosa (Sunkara et al., 2012; Jäger et al., 2018), and producing bioactive metabolites like bacteriocins that directly inhibit the proliferation of harmful bacteria such as *E. coli* (Bu et al., 2023)Furthermore, the production of lactic acid creates an acidic intestinal environment that suppresses pathogen growth while promoting a beneficial microbiota, as previously noted.

***Conclusion*:** The results of this study demonstrate that dietary supplementation with *L. plantarum* significantly improves growth performance and feed efficiency in chickens. By enhancing immune function and reducing susceptibility to pathogens, notably *E. coli*, it fosters better overall health. The marked reduction of *E. coli* in the intestines and excrement confirms its efficacy. These findings suggest that *L. plantarum* is a potent natural alternative to antibiotics, promising to enhance the safety and sustainability of poultry farming.

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***Conflict of interest*:** The authors declare that there are no conflicts of interest.

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***Policy addressed*:** National and Global Policies on Reducing Antibiotic Use in Livestock; Policies on Safe and Sustainable Food Production; Livestock Health and Biosecurity Policies; Sustainable Agriculture Policies.

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